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Microvascular Alterations in the Lymph Node During the BCG-induced Immune Response

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Summary

Previous studies have shown significant microvascular changes during the evolution of the immune response. In order to assess the BCG-induced microvascular alterations in the lymph node, we injected the left foot pad of 23 rabbits with 1.6 x 10^6 live BCG/kg weight; the right side served as control. Following a period of 1 to 50 days, microangiography and histology of the popliteal lymph nodes were carried out. On the side where BCG was injected, the lymph nodes initially showed a moderately increased cortical, and later, a markedly increased medullary hypervascularity. Histologically, there was mixed mononuclear cellular infiltration followed by granuloma formation. This latter was temporally related to the florid medullary hypervascularity. No significant changes were present on the control side. The hypervascularity induced by BCG was more intense and longer lasting than that seen with other antigens such as Typhoid 0 or bovine serum albumin.

The results indicate a prominent vascular component in the BCG-induced primary immune response within the regional lymph node.

Although the therapeutic efficacy of immunologic agents in the treatment of neoplasms is still unproven, the mechanisms by which they act have drawn considerable investigative interest. The immunotherapeutic agent which thus far has received the greatest attention is BCG. BCG's effect is most likely the result of a non-specific cellular immune response (1, 2, 3), as well as its acting as an adjuvant to induce tumor-specific systemic immunity (4, 5). Previous studies with other antigens have shown lymph node microvascular changes to occur concomitantly with the cellular immune response (6, 7, 8). The purpose of this study was to investigate BCG's effect on the microvasculature of a regional lymph node. The assumption was that BCG might evoke a strong and sustained hypervascular response, which may be related to its effectiveness as a potent immunologic agent.

Materials and Methods

BCG was obtained from the Pasteur Institute, Paris, France in two cc ampules with a concentration of 0.8 ± 0.4 x 10^8 live, attenuated bacilli per cc.

To establish the optimal BCG dosage for producing the maximal immune response, six, three kg New Zealand white rabbits had BCG injected in varying doses into their left foot pads; their right legs served as controls. The doses injected included 0.8 x 10^6, 1.6 x 10^6 and 3.2 x 10^6 bacilli per kg. Three days following inoculation, the rabbits were anesthetized with one to two cc's intravenous Nembutal. A P.E. 190 catheter was placed in the abdominal aorta and a P.E. 240 catheter in the inferior vena cava. Two liters of Dextran solution (Gentran, Travenol) perfused the animal at 140 mm Hg constant pressure via the aortic catheter, resulting in a bloodless venous effluent. One liter Micropaque solution (10% by volume in Dextran) was then administered at the same pressure via the same aortic catheter. The Micropaque remained suspended throughout by constant magnetic stirring.

Both the experimental left popliteal lymph node and the right control node were dissected free, weighed, and stored for 48 hours in 15% formaldehyde, then processed by alternating microangiographic (200 microns) and histologic (6 microns) sections. The former were radiographed at 15 kVp and 15 mA

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using a beryllium window X-ray tube. The histologic sections were stained with hemotoxylin and eosin.

Histologic evaluation of the lymph nodes obtained from the six pilot animals showed the maximal response to occur following innoculation of 1.6×10^6 bacilli/kg body weight. This dosage was used for all animals included in the present study. No greater cellular infiltration was subjectively discerned for higher doses of BCG.

Twenty-three experimental animals were treated as described for the pilot study, being sacrificed at intervals from one to fifty days following BCG innoculation. Histologic sections of control and experimental lymph nodes were evaluated by one of the authors (WMB) for cortical invasion of inflammatory cells, lymphoid infiltration of the medulla, and granuloma formation. Specimens were rated 0–+++ for the presence of these findings (0 = not present; + = sparse presence; ++ = moderately present; +++ = floridly present). The accuracy of his evaluations was confirmed by the independent examination of a second pathologist (Dr. *Franz von Lichtenberg,* Harvard University Medical School). A similar rating system was employed by the other two authors (BJH, PGH), who independently evaluated the microangiographic sections for cortical and medullary hypervascularity (increased number of small vessels) and vascular stretching. For both histologic and micro-angiographic evaluations, there was generally good agreement on the extent of alterations. When disparities did occur, they were resolved by consultation.

Results

Lymph Node Weight – Uniformly, there was an increase in experimental lymph node weight when compared with the contralateral control node.

For Days One through Eight, the experimental/control weight ratio ranged from 1.2 to 3.7 with a mean of 2.2. For Days 11 to 20, the range was 3.1 to 10.3 and the mean ratio was 5.1. Experimental node weight returned toward normal on Days 23 through 50 with ratios ranging from 1.2 to 2.4 with a mean of 1.9.

Histologic Alterations – The early polymorphonucleocyte infiltration was transient, rapidly giving way to a diffuse and more prolonged mononuclear hypercellularity (Figure 1a, 1b). Germinal center formation was sporadic and not a prominent feature of the histology. A cortical histiocytosis became evident around Day Four and progressed in extent, eventually evolving into well-defined granulomata by the seventeenth day following BCG inoculation (Figure 2). Granulomata were observed in all experimental lymph nodes subsequent to this time period.

Until Day Three, contralateral control nodes showed minor (0–+) cellular changes similar to, but considerably less extensive than those seen on the experimental side. Following this time, no inflammatory involvement of control lymph nodes was discerned. Granulomata were not observed in control nodes.

Microvascular Alterations – A normal lymph node microangiogram is depicted in Figure 3. An early mild cortical hypervascularity (F (Figure 4) was supplanted, beginning on Day Three, with a more profound medullary hypervascularity (Figure 5). The medullary vessels were extremely fine and profuse, becoming even more prolific and disordered by Day 17 (Figure 6). Cortical vessels became increasingly stretched during this period and occasional cortical extravasation occurred. The vascular changes seen in experimental nodes diminished following Day 23, however, mild stretching of cortical vessels peristed through the fiftieth day.

During the first three days following BCG innoculation, several of the contralateral control lymph nodes showed mild degrees of cortical hypervascularity. Control nodes were otherwise uniformly normal in their microvascular patterns.

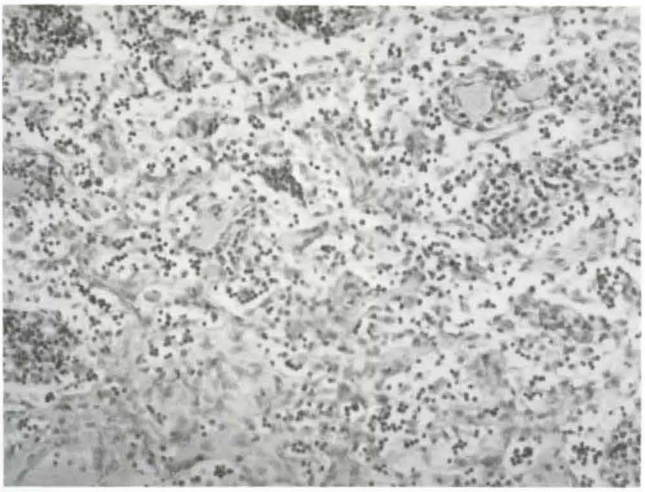

Fig. 1a 50 x photomicrograph of lymph node medulla three days following BCG injection, showing a mixed mononuclear cellular infiltration

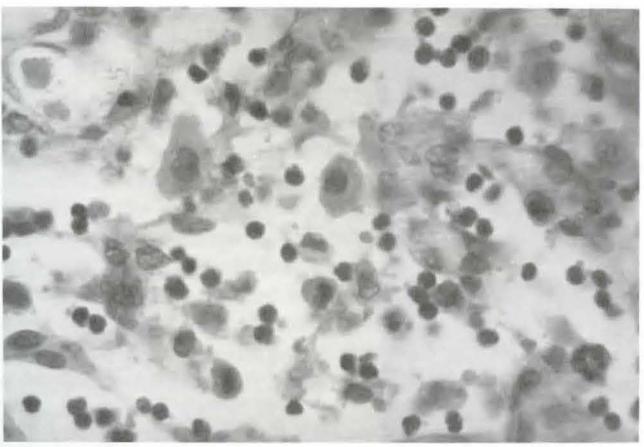

Fig. 1b 200 x photomicrograph depicting an area of the medulla shown in Figure 1a

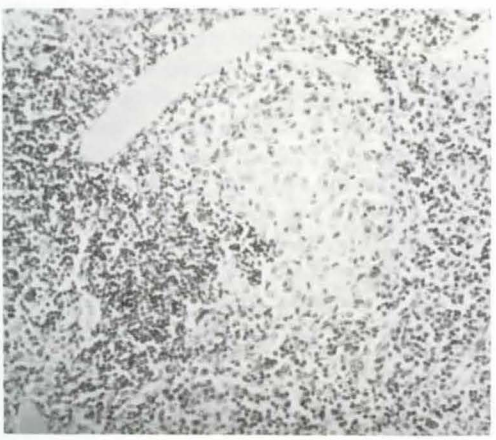

Fig. 2 50 x photomicrograph of a lymph node 20 days following BCG innoculation, showing a developing granuloma

Discussion

Our results indicate that subcutaneous injection of BCG into rabbit foot pads evokes a significant microvascular alteration in lymph nodes draining the inoculation site. This vascular reaction is temporally related to the development of a cellular immune response. The pattern of histologic changes we observed is similar to that described by *Adams*, except for the earlier appearance of granulomata in his guinea pigs following BCG administration (9). The floridity of cellular infiltration and edema is reflected in the weight gain of experimental lymph nodes when compared with contralateral controls. The observed stretching of cortical vascular arcades is probably also resultant of this phenomenon.

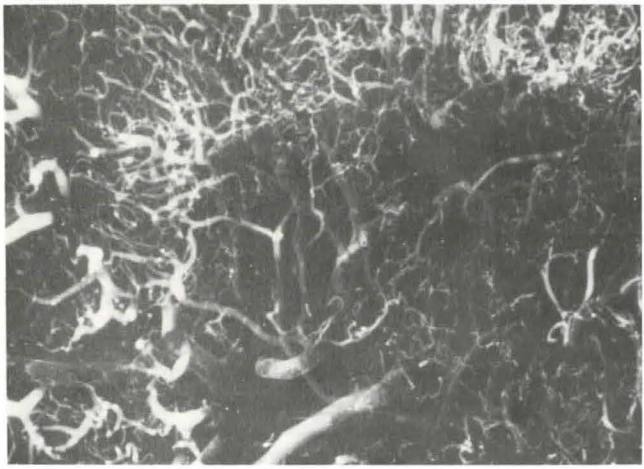

Fig. 3 60 x microangiogram of a normal lymph node

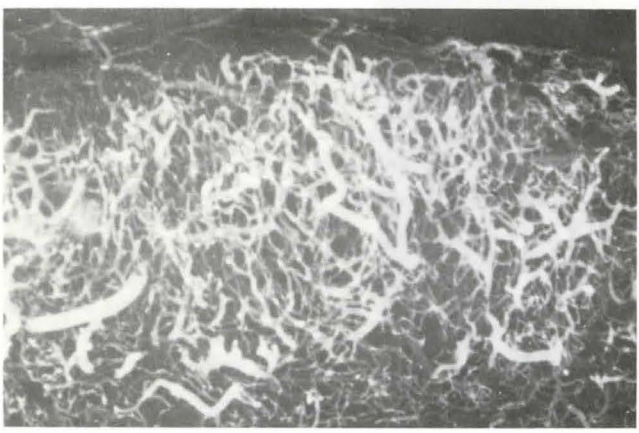

Fig. 4 60 x microangiogram of lymph node cortex one day following BCG injection. There is a mild cortical hypervascularity

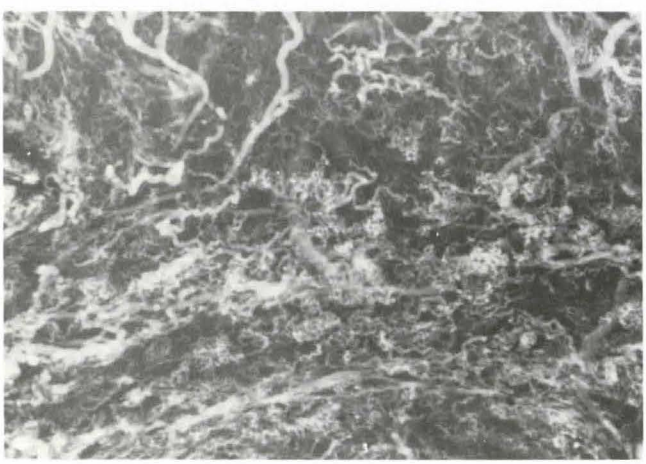

Fig. 5 60 x microangiogram three days after BCG injection depicting a florid medullary hypervascularity

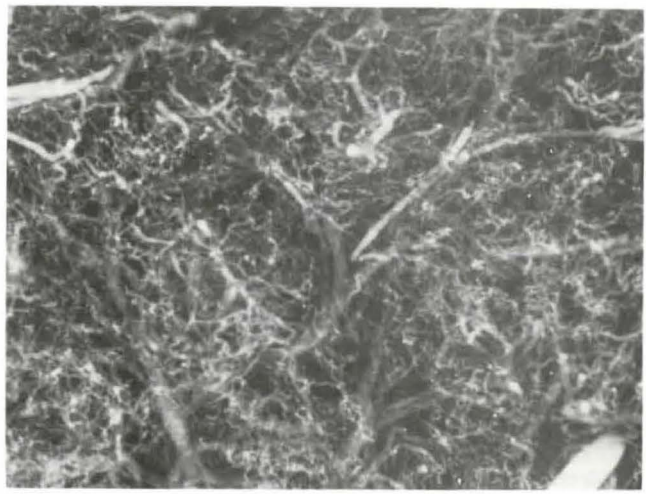

Fig. 6 60 x microangiogram 17 days following BCG innoculation. The medullary vasculature is greatly increased

The medullary microvascular response appeared biphasic, reaching its peak hypervascularity at around three days and again at 14–17 days. Interestingly, this appears to correlate well with the times of first polymorphonuclear, and later, histiocytic cellular infiltrations. The rapid diminution in medullary hypervascularity following the establishment of granulomata suggests that the vascular reaction is causally related to the cellular immune response. These microvascular changes are particularly interesting with respect to the hypothesized mechanisms of BCG-induced anti-neoplastic activity. BCG's efficacy is generally attributed to a combination of nonspecific and specific immune mechanisms (10, 11). The former appears related to the induction of a chronic inflammatory response, with perivascular infiltration of polymorphonuclear and mononuclear leukocytes (11), eventually evolving into mature granulomata (12). Activated macrophages, stimulated lymphocytes (10, 11), and perhaps "natural killer cells" (13) may all participate in this phenomenon, which is doubtlessly facilitated by the increased vascular access we have described. In addition, the hypervascular response may augment the proliferation of lymphocytes by spreading macrophage-secreted humoral factors (14) or by stimulating tumor specific immunity by trapping thymus dependent lymphocytes at the tumor site (15). Finally, BCG-induced, nodal vascular alterations may

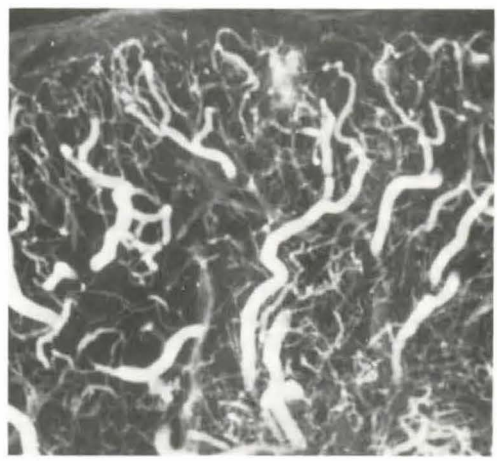

Fig. 7 60 x microangiogram of lymph node cortex, 17 days after BCG administration. Cortical microvasculature is mildly stretched and there is a focus of extravasation

enhance immune mechanisms by producing an unfavorable milieu (10, 11). Indeed, Micropaque extravasation – which we observed during the later period of extreme hypervascularity (Days 14–20) – indicates diminished vascular integrity at this time (Figure 7).

We have demonstrated that considerable and long lasting hypervascular alterations occur in lymph nodes undergoing a strong, BCG-induced, cellular immune reaction. These changes are more intense and sustained than those that

we have observed to occur with other antigens such as Typhoid 0. The temporal relationship between microvascular and histologic changes suggests that these vascular alterations may indeed be important in the genesis of the immune response.

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